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From: Schmidt, Mary
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RIBOZYME MEDIATED CLEAVAGE OF HEPATITIS B VIRUS SURFACE ANTIGEN mRNA

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The hepatitis B virus surface antigens (HBsAg) which form the envelope of the viral particle are encoded by three overlapping genes, namely the pre-S1, pre-S2 and S genes. The major HBsAg protein is the 226 amino acid S gene product derived from a 2.1 Kb subgenomic RNA. Since HBsAg is vital for the production of infectious virus, the cleavage of this RNA by ribozymes could form the basis for a novel therapy. There are nine canonical "GUC" hammerhead ribozyme cleavage sites in the open reading frame of the S mRNA. We have synthesized hammerhead ribozymes against all nine sites and assessed the ability of these ribozymes to cleave both a 0.8 Kb fragment and the full length mRNA. Of the nine ribozymes only two could cleave the mRNA at 37°C in 10 mM $MgCl_2$ as assessed by the identification of radiolabeled cleavage products on a polyacrylamide gel. However, upon repeated heating and cooling of the enzyme and substrate (three rounds of heating to 65°C followed by snap cooling and then incubating at 37°C), the expected cleavage products were seen in all cases. This demonstrates that all nine ribozymes are capable of cleaving the mRNA if higher order structures are disrupted by heating and cooling. The two sites that were cleaved by the ribozymes without thermal denaturation were not in regions that were predicted to be unstructured by computer simulated folding programs based on least energy structure. We have cloned the cDNAs for these ribozymes into eukaryotic expression vectors under the control of Rous sarcoma virus LTR promoter. These constructs were transfected into HBsAg-expressing hepatoma cells by using liposomes or by complexing the plasmid to asialoorosomucoid for specific uptake via the asialoglycoprotein receptor. Expression of the ribozymes in the cells caused a rapid decrease in the production of HBsAg.

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The Observation on the Antiviral Effect of Ribavirin and Combining with Interferon in Patients with Chronic Hepatitis B

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In this paper, the recent and followed-up antiviral effect was observed in 23 patient and chronic hepatitis B. Among them, the group of ribavirin combining with interferon (Group I) was 12 cases, and the group of ribavirins (Group II) was 11 cases. The contrast group, who was treated without antiviral drug was 12 cases. The recent t negative percentage of HBeAg, DNAP, HBVDNA was 41.6%, 55.5% 28.5% in group I, 45.5% 100%, 33.3% in group II, and 8.3%, 16.6%, 8.3% in contrast group. The followed-up negative percentage of HBeAg, DNAP was 70%, 83.7% in group I, 60% 100% in groupII, and 8.3%, 50% in the contrast group. From the synthetical antiviral effect, the recent valid rate in group I, IIand contrast was 50%, 72.1% and 16.6% the followed-up valid rate was 70%, 55.5% and 8.3% respectively. No side-effect appeared. All patients finished the course.